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Leucoagaricus dacrytus – a new species from New Jersey, U.S.A.

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Abstract — *Leucoagaricus dacrytus* (*Agaricaceae*) is described as new from an old-growth *Quercus rubra* forest in New Jersey, U.S.A. This is a relatively small, brown species exuding golden drops over its surface, with a pileus with cobwebby patches (a cutis-like pileus covering), narrowly clavate cheilocystidia, and oblong-amygdaloid spores. It is close to the European species *La. tener*, from which it differs in the slightly smaller spores and in nrITS sequences.

Key words — biodiversity, North America, taxonomy

Introduction

Several unknown lepiotaceous fungi were discovered during long-term mycological research in the Oakmoss Mycological Preserve, a forest with old-growth *Quercus rubra* and many secondary trees (*Fagus, Betula, Cornus, Acer, Carya, Prunus*, and sassafras) in New Jersey; the specimens were sent to the first author for identification. One of them is a striking brown species with golden droplets on the surfaces of the basidiocarps. A literature search (starting with Lincoff 1991, Bessette et al. 1997, moving to Murrill 1914, Kauffman 1924, and Smith 1954, 1966) did not result in a fitting name. Here it is described as a new species, based on the morphology and the nrITS sequences, and it is compared with *Leucoagaricus tener* (P.D. Orton) Bon from Europe, and species with similar general morphology from other parts of North America and Europe.

Material & methods

Macroscopic descriptions were based on the photos and notes provided by the second author. Standard methods for describing basidiocarps were applied, using the

terminology of Vellinga & Noordeloos (2001). Colour codes are according to the Online Auction Color Chart™, indicated by 'oac' before a number. Microscopical observations were made on dried material. The notation [115,6,5] indicates that measurements were made on 115 spores in six samples in five collections. At least 15 spores were measured per collection. The lamellar characters and spore shape and size were observed in Congo Red in 10% ammonia followed by ammonia only, and the pileus covering was observed in 10% ammonia. The following abbreviations are used: L for number of lamellae, l for number of lamellulae between two lamellae, avl for average length, avw for average width, Q for quotient of length and width, and avQ for average quotient. The abbreviation *L.* is used for *Lepiota*, *La.* for *Leucoagaricus* and *Lc.* for *Leucocoprinus*. All collections are in UC. Herbarium abbreviations are according to Holmgren & Holmgren (1998). The Latin description of the new species has been deposited in MycoBank.

DNA was extracted from dried material using a Qiagen DNeasy® Blood and Tissue kit (Qiagen, Valencia, CA, USA). The nrITS region was amplified with the ITS-1F/ITS-4 primer set with an MJ PTC-100™ thermocycler (Applied Biosystems, Foster City, CA, USA) under conditions previously described (Gardes & Bruns 1993). PCR products were cleaned using 0.5 µl of ExoSAP IT (USB Corp, Cleveland, OH, USA) per reaction and cycled at 37°C for 45 min, followed by 80°C for 15 min. Sequencing was performed using Big Dye chemistry and an ABI PRISM 3100 Genetic Analyzer (both from Applied Biosystems, Foster City, CA, USA). Sequences were edited and contigs assembled using Sequencher 4.2.2 (Gene Codes Corporation, Ann Arbor, MI, USA). Newly produced sequences were deposited in GenBank, and their accession numbers are listed with the collections and all accession numbers are given in Fig. 3. American *Leucoagaricus* species with brown to black cobwebby patches on the pileus surface were chosen for sequence comparisons, mainly from species in the *L. atrodisca* species complex in California, but also based on BLAST searches in GenBank (Altschul et al. 1990). The sequences were aligned with the program MAFFT version 6 (Katoh et al. 2002, Katoh & Toh 2008). For the phylogenetic analyses the Maximum Parsimony option in PAUP* v4 (Swofford 2002) was used. *Chlorophyllum rachodes* (Vittad.) Vellinga and *Leucoagaricus americanus* (Peck) Vellinga were chosen as outgroup. The analyses were only performed to determine whether the sequences matched sequences of previously sequenced species and collections.

Taxonomy

Leucoagaricus dacrytus Vellinga, **sp. nov.** Figures 1 & 2

MycoBank MB516742

Leucagarico tenero *similis, sporis nonnihil minoribus (i.e. 5.9–7.4 µm longis 2.9–4.1 µm latis, in medio 6.3–6.8 µm longis 3.5–3.9 µm latis) plus quam 50 basibus ceteris in ITS1 differt.*

Holotype: "U.S.A., New Jersey, Hunterdon Co., Lebanon, Oakmoss Mycological Preserve, R.B. Balsley, 11 Sept 2006 (UC)."

Etymology—from the Greek δακρυτος, tearful, because of the presence of drops on the basidiocarps.

Pileus 10–33 mm, when very young paraboloid to hemispherical with inflexed margin, expanding to plano-convex, and finally applanate with low and broad

Fig. 1. *Leucoagaricus dacrytus* — Habitus (from Balsley, 12 July 2006).

umbo, when young almost completely dull brown (oac702) except for a marginal, lighter zone, later only brown at umbo, and very light at margin, covered in small fibrillose-cobwebby patches to tufts, dense at centre, thinner at margin and there showing off-white background, on drying slightly sulcate for up to 5 mm at margin, covered with scattered drops, changing from golden yellow (oac856) to brown with age. Lamellae, L = 45–55, l = 1(–3), moderately crowded to crowded (2–3/1 mm, measured at pileus margin), free, relatively close to stipe, off-white to pale cream coloured, with concolourous not obviously cystidiose edge. Stipe 20–50 × 1.5–3.5 mm cylindrical and slightly wider, 3–5 mm, at utmost base, off-white above annulus, below annulus off-white changing to pale yellow with age, slightly darker when scratched, when fresh covered in pale yellow to yellow (oac856) drops, sometimes with basal white tomentum, hollow, with white mycelial cords at base. Annulus an ascending funnel with small flaring part, off white, with golden drops on underside. Smell none.

Basidiospores [105,6,5] in side view 5.9–7.4 × 2.9–4.1 µm, avl × avw = 6.3–6.8 × 3.5–3.9 µm, Q = 1.5–2.15, avQ = 1.7–1.85, oblong-amygdaliform, with rounded apex, in frontal view oblong-ovate, with guttule, thick-walled some with a hint of an apical germ pore, young spores in particular congophilous, cyanophilous, dextrinoid, when young clearly metachromatic in Cresyl Blue, later less evidently so. Basidia 13.5–27 × 6.0–8.5 µm, with 4 sterigmata, without clamp connection. Lamella edge sterile, with a band of cystidia. Cheilocystidia 22–50 × 6–13 µm, narrowly clavate to subutriform, some clavate, some slightly strangulated, subtly variable in shape, slightly thick-walled, and colourless. Pleurocystidia absent. Pileus covering cutis-like, made up of strands of hyphae with up to 5 coloured elements in a row; terminal elements, slightly differentiated and wider than penultimate elements, 27–95

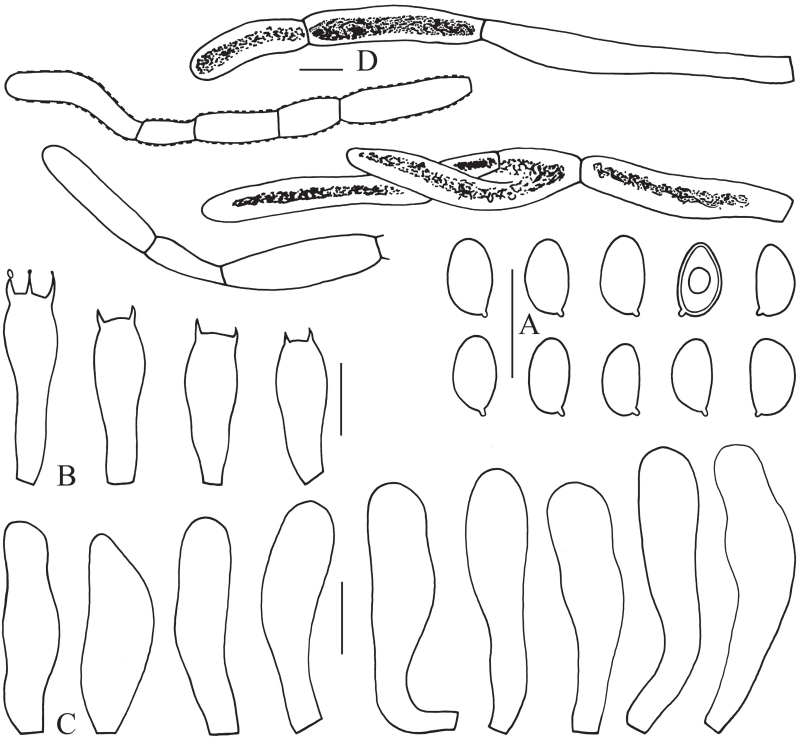

Fig. 2. *Leucoagaricus dacrytus* — A. spores, B. basidia, C. cheilocystidia, D. pileus covering elements (all from Balsley, 30 Aug. 2007). Scale bars are 10 µm.

× 5–12.5(-23) µm, with rounded apex; pigment brown, intracellular in big blob, or parietal and incrusting, in all elements, also the terminal ones. Clamp connections absent.

Habitat & distribution — In small groups, on decayed wood, most likely from *Quercus rubra*, in a deciduous forest (old growth *Quercus rubra* plus various other deciduous trees); so far only known from one spot at the type locality in New Jersey. July–Sept.

Collections examined—U.S.A., **New Jersey**, Hunterdon Co., Lebanon, Oakmoss Mycological Preserve, 12 July 2006, R.B. Balsley (nrITS GU903308); ibidem, 8 Sept 2006, R.B. Balsley; ibidem, 11 Sept 2006, R.B. Balsley (nrITS GU903309)(Holotype, UC); ibidem, 26 Aug 2007, R.B. Balsley; ibidem, 30 Aug 2007, R.B. Balsley.

Discussion

Leucoagaricus dacrytus is characterized by brownish tinges in the pileus, the golden drops exuded on the basidiocarp surface, and microscopically by the

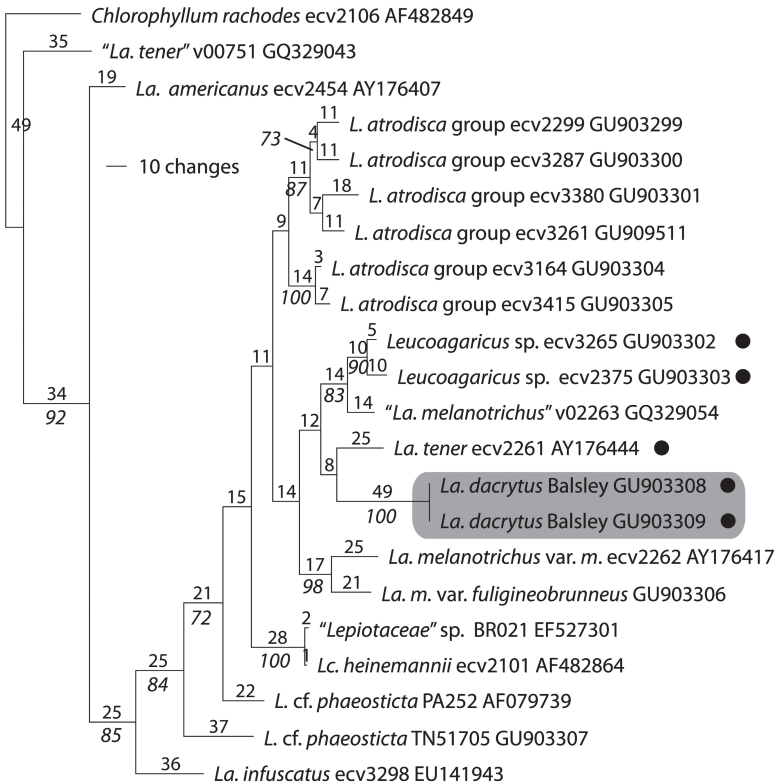
Chlorophyllum rachodes ecv2106 AF482849
"La. tener" v00751 GQ329043
La. americanus ecv2454 AY176407
10 changes
L. atrodisca group ecv2299 GU903299
L. atrodisca group ecv3287 GU903300
L. atrodisca group ecv3380 GU903301
L. atrodisca group ecv3261 GU909511
L. atrodisca group ecv3164 GU903304
L. atrodisca group ecv3415 GU903305
Leucoagaricus sp. ecv3265 GU903302
Leucoagaricus sp. ecv2375 GU903303
"La. melanotrichus" v02263 GQ329054
La. tener ecv2261 AY176444
La. dacrytus Balsley GU903308
La. dacrytus Balsley GU903309
La. melanotrichus var. m. ecv2262 AY176417
La. m. var. fuligineobrunneus GU903306
"Lepiotaceae" sp. BR021 EF527301
Lc. heinemannii ecv2101 AF482864
L. cf. phaeosticta PA252 AF079739
L. cf. phaeosticta TN51705 GU903307
La. infuscatus ecv3298 EU141943

Fig. 3. Phylogram based on parsimony analyses of the nrITS region of a selection of *Leucoagaricus* and *Leucocoprinus* species with brown to black cobwebby scales made up of repent hyphae on the pileus surface. The one and only most parsimonious tree is presented, based on 211 informative characters. The numbers above branches refer to the number of changes, those below, in italics, to bootstrap values. The symbol ● indicates the presence of exudates. The newly described species *La. dacrytus* is highlighted. *Chlorophyllum rachodes* and *La. americanus* are outgroup taxa.

relatively small spores, the narrowly clavate cheilocystidia, and the cutis-like pileus covering with intracellular and incrusting pigments.

It is close, both morphologically and molecularly, to the European species *La. tener*, which differs in having smaller basidiocarps, slightly longer and wider spores, and more cylindrical cheilocystidia (Orton 1960, Uljé 1984, Vellinga 2001). These morphological differences are small and subtle. The nrITS sequences of both taxa are quite different (Fig. 3), with at least 50 different base pair positions in the ITS1 alone.

Drops on the basidiocarp surface are not restricted to these two species, but are found in many species in the *Leucoagaricus/Leucocoprinus* clade of the *Agaricaceae*, such as *Leucocoprinus cepistipes* (Sowerby) Pat. (sensu Lange 1935), and *Lc. lacrymans* T.K.A. Kumar & Manim. The former often has drops on its greyish pileus, stipe, and annulus, but the spores have a germ pore, and the cheilocystidia are big with an apical excrescence (e.g. Vellinga 2001). *Leucocoprinus lacrymans* from India also has spores with a germ pore, stains reddish when damaged, and has long, cylindrical cheilocystidia (Kumar & Manimohan 2004).

Neither the origin of the drops, nor the composition of them is known for this group of fungi. Many polypore species exude drops during the growing period, and it is known that *Pseudoinonotus dryadeus* (Pers.) T. Wagner & M. Fisch. exudates have a negative effect on the growth of gram positive bacteria (Blackwell & Adams 1985).

Other species that bear some resemblance to the presently described species are the following: *Leucoagaricus brunneocingulatus* (P.D. Orton) Bon, known from the United Kingdom (Orton 1960) and Italy (Migliozzi & Perrone 1991), lacks drops, is red-brown on the pileus, and has a brown-rimmed annulus. The brown-scaled *La. brunneosquamulosus* P. Mohr & Dähncke, described from the Canary Islands, is clearly different because of the spores with a distinct germ pore and upright cylindrical to narrowly lageniform terminal elements in the pileus covering, and the narrow, cylindrical cheilocystidia (Mohr & Ludwig 2004). *Leucoagaricus infuscatus* Vellinga is another brown-squamulose species, with a cutis-like pileus covering; its brown pileus centre contrasts with the white background, and the absence of drops and the narrowly clavate to almost capitate cheilocystidia differentiate it from *La. dacrytus* (Vellinga 2007).

Sequences of species with comparable, but almost black pileus coverings, such as *L. atrodisca* Zeller, *La. melanotrichus* (Malençon & Bertault) Trimbach and *Lc. heinemannii* Migl., have been added to the group for comparison with *La. dacrytus* and *La. tener*, along with some unnamed species from California. Grey-brown species with drops on the basidiocarps are indicated in the resulting hypothetical phylogeny of Fig. 3. This group is species rich, with representatives all over the world, and desperately in need of morphological revision.

The species described and depicted by La Chiusa (1999) as *La. tener* is different from the original as described by Orton (1960); it lacks drops, the pileus covering is made up of velvety patches, not the cobwebby patches of *La. tener*, and its nrITS sequence (GenBank accession number GQ329043) differs significantly from the Dutch collection of *La. tener* (GenBank accession number AY176444; fig. 3). Likewise, Migliozzi & Coccia's interpretation of *La. tener* differs from Orton's concept (Migliozzi & Coccia 1990, Vellinga 2001), though an nrITS sequence is not available for their specimens.

It is possible that *La. dacrytus* was described under a different name from North America, although the presence of droplets on the basidiocarps is not mentioned in any of the species descriptions, nor in the keys to the *Lepiota* species provided by Murrill (1914) and Kauffman (1924). Murrill included in his key all the species known at that time, and described by Peck, Morgan and others. The presence of droplets could have been noticed by the collector, but interpreted as unimportant or the result of external factors, such as rain. Reid (1995) did not report the presence of drops on *La. tener* basidiocarps, though Orton (1960) in the original description, did notice them. Recently, the presence of drops was omitted in the description of *L. furfuraceipes* Han C. Wang & Zhu L. Yang, a new species from Yunnan, China, and northern Thailand (Wang & Yang 2005). This species is common in northern Thailand, where the first author has repeatedly collected it; drops were always present on stipe and annulus, leaving dark spots on the annulus margin.

This paper hopefully will draw attention to the taxonomic significance of exudate drops on the basidiocarps of lepiotaceous fungi, and will also provide a stimulus to investigate this group of beautiful fungi in the eastern parts of North America.

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